Full Length Research Paper

DPPH radical scavenging and lipoxygenase inhibitory effects in extracts from *Erythrina senegalensis* (Fabaceae) DC

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Received 4 October, 2015; Accepted 9 November, 2015.

*Erythrina senegalensis* DC (Fabaceae) is a plant used in traditional medicine in Burkina Faso (West Africa) to cure several diseases such as malaria, rheumatism, abdominal pain, fibroma and infections which are always accompanied by oxidative stress. This study aimed to highlight the antioxidant activities in dichloromethane (DCM) and ethyl acetate (EtOAc) extracts of *E. senegalensis* stem bark and roots. We used the 1, 1 diphenyl-2-picryl hydrazyl (DPPH) radical scavenging test and the 12-lipoxygenase I-B inhibitory method. We previously characterized the phytochemical groups by thin layer chromatography and colored reactions in tubes. The extracts in DCM (IC⁵₀-root: 5.18 ± 0.06 and IC⁵₀-bark: 5.76 ± 0.68) showed strong scavenging activity. However, as a 12-lipoxygenase inhibition, the DCM extracts were almost inactive. The EtOAc extracts from root (IC⁵₀-LOX: 7.21 ± 2.31; IC⁵₀-DPPH: 7.27 ± 0.13) and from stem bark (IC⁵₀-LOX : 4.95 ± 1.12; IC⁵₀-DPPH : 11.4 ± 1.3) presented both the radical scavenging and the 12-lipoxygenase inhibitory effects. Polyphenols (flavonoids, tannins), steroids and terpenoids characterized in all extracts may be involved in the observed 12-lipoxygenase inhibition and radical scavenging.

**Key words:** *E. senegalensis*, antioxidant, 12-lipoxygenase I-B inhibitory.

INTRODUCTION

*Erythrina senegalensis* DC (Fabaceae) is a plant used in traditional medicine in Burkina Faso (West Africa) to cure several diseases such as malaria, rheumatism, abdominal pain, fibroma (Nacoulma et al., 1999), skin...
diseases and amenorrhea in Mali (Togola et al., 2008). Aqueous stem bark extracts are effective against venereal and pulmonary infectious diseases (Iwu, 1993). The antioxidant properties of extracts from E. senegalensis are rarely assessed while phytochemicals are good antioxidants because they are able to scavenge free radicals or inhibit oxidative enzymes such as cyclooxygenase and lipoygenase. And then, phytochemicals are also less mutagenic and teratogenic than the synthetic antioxidants.

Lipoxygenases (LOXs EC1.13.11.12) are nonheme iron-containing dioxygenases that catalyze the formation of corresponding hydroperoxides from polyunsaturated fatty acids such as linoleic and arachidonic acids. They are mainly called 5-, 12-, and 15-LOX based on their ability to insert molecular oxygen at the 5-, 12-, or 15-carbon atom of arachidonic acid (Wisastra and Dekker, 2014). LOX enzymes expressed in immune, epithelial, and tumor cells are an important source of reactive oxygen species (ROS) that display a variety of functions, including inflammation, skin disorder, and tumorigenesis (Mashima et al., 2015). So, implicated in the pathogenesis of inflammatory and hyperproliferative diseases, the LOXs represent potential targets for pharmacological intervention.

Free radicals increase in the body during inflammation, exercise or after exposure to exogenous sources such as pollution, smoking, certain medications and radiations (Lobo et al., 2010). An excess of oxidative stress can lead to the oxidation of lipids and proteins, which is associated with changes in their structure and functions (Lobo et al., 2010). Free radicals may oxidize and modify the DNA’s genes or the cellular regulatory proteins and lipids leading to many metabolic and cellular disturbances such as cancer, asthma, atherosclerosis, cataract and inflammatory diseases (Lobo et al., 2010).

The antioxidant system of the body, essentially enzymatic (superoxide dismutase, catalase, glutathione peroxidase) is often swamped and free radicals become dangerous for the health without an appropriate treatment. For all these reasons, it is very important to find vegetal antioxidant compounds from E. senegalensis that are able to reduce oxidative damage. The aim of this study is to characterize main phytochemical groups in the extracts of E. senegalensis and to show their free radical scavenging and 12-lipoxygenase inhibitory effects.

**MATERIALS AND METHODS**

**Plant materials**

Fresh roots and stem bark of *Erythrina senegalensis* (Fabaceae) DC, after locating the plant (30P0641573 UTM132 7922), were collected in June 2009 from their natural habitat in Saponé, at 50 km from Ouagadougou, Burkina Faso (West Africa). The specimen was certified by Dr. Souleymane GANABA, Department of Forestry of the National Centre for Scientific Research and Technology of Ouagadougou. A voucher specimen was deposited at Burkina National Herbarium (HNBU) and attributed No. 8709. Collected plant materials were dried at room temperature under shade to prevent the direct effect of sun. The resultant dried plant parts were individually reduced to powder with mortar and pestle, sieved and kept in a clean dried cupboard before use.

**Extraction of plant materials**

The extraction of phytochemicals is made by successive exhaustion with increasing polarity solvents. Three hundred grams of powder (300 g) were initially defatted with ether, dried and successively exhausted with dichloromethane and ethyl acetate. The exhaustion of the drugs by a solvent is continued until the percolating liquid becomes limpid. Each liquid obtained by filtration was freeze-dried in a rotavapor to yield a solid residue. Appropriate concentrations of the extract were made and used in experiments. Dried plant drugs powder was weighed (M). After a complete exhaustion, the dry extract obtained from the rotavapor was also weighed (m).

Extraction r (%) performance is given by the formula:

\[ r = \frac{[m / M]}{100} \]

**Phytochemical screening**

Standard screening colorimetric tests of the extract were carried out for various constituents according to Ciulei et al. (1982) and completed by thin layer chromatography. The extracts were screened for the presence of alkaloids, flavonoids, saponins, coumarins, tannins, steroids and triterpenes.

**DPPH (1, 1-diphenyl-2-picryl hydrazyl) free radical scavenging activity**

The test was carried out according to Kim et al. (2003) with a slight modification. The reaction mixture contained test sample (extract or quercetin) in dimethyl sulfoxid and DPPH in methanol (101 µM, Sigma, Germany). The reaction mixture was incubated at 37°C for 30 min. The absorbance was measured at 520 nm. The percentage of radical scavenging activity was determined by comparison with a DMSO-containing control. Inhibitory concentration 50 (IC50) values represented the concentration of compounds to scavenge 50% of DPPH radicals. Quercetin was used as a positive control. All the chemicals used were of analytical grade (Sigma, Germany).

**In vitro lipoxygenase inhibition assay**

Lipoxygenase inhibiting activity was measured by the spectrometric method developed by Lyckander and Malterud (1992) and adapted according to our working conditions. Lipoxygenase (1.13.11.12) type I-B and linoleic acid were purchased from Sigma (Germany). All other chemicals were of analytical grade. 400 µL of sodium borate buffer (0.2M, pH 9.0) containing lipoxygenase (167 U/mL) and 100 µL of test compound solution were mixed and incubated for 10 min at 25°C. The reaction was then initiated by the addition of 500 µL linoleic acid (substrate) solution. This reaction led to the formation of (9Z, 11E)-13-hydroperoxyoctadecadienoic acid, and the change of absorbance at 234 nm for 10 min. Test compounds and the control were dissolved in methanol. Measurements of increase in absorbance at 234 nm for 30–90 s give extract inhibitory activities. All the reactions were performed in triplicate.

**Treatment of data**

All experiments were performed in quadruplicate (n = 4). The data
Table 1. Extracts obtained by successive extraction with different increasing polarity solvents.

<table>
<thead>
<tr>
<th>Plant material</th>
<th>% of extraction per solvent</th>
<th>Dichloromethane</th>
<th>Ethylacetate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stem bark</td>
<td></td>
<td>4.24</td>
<td>1.16</td>
</tr>
<tr>
<td>Roots</td>
<td></td>
<td>1.56</td>
<td>0.30</td>
</tr>
</tbody>
</table>

were given as mean ± SEM. Values of 50% inhibitory concentration (IC$_{50}$) were determined from the dose curves / effects obtained using the PRISM version 5.0 software.

RESULTS

Yields of extractions

Successive exhaustion by permeation of plant to obtain extracts masses whose catches in tests are presented in Table 1. It showed that by percolation of the plant, the masses of extracts vary according to the solvents used and according to the parts of the plant. *E. senegalensis* stem bark was the richer source of lipophilic extracts, more than roots. Dichloromethane gave higher yields of extraction than ethyl acetate extracts.

Colored reactions in tubes

Steroids, triterpenes and coumarins were detected in all the parts of the plant. Flavonoids and tannins were found more in stem bark than in roots (Table 2).

Thin layer chromatography

TLC (Figure 1) has indicated a predominance of catechic tannins over gallic tannins in all the parts of the plant. Flavonoids were the most abundant in the stem bark extracts. Root extracts contained more phenolic acids than flavonoids. Triterpenes and steroids have been characterized in all the parts of plant.

Radical scavenging activity in extracts of *E. senegalensis*

Extracts have shown dose-dependent effects, as in Figure 2. DCM extracts of root and stem bark have globally shown the best dose-dependent radical scavenging effect (IC$_{50}$-root: 5.1 µg/mL and IC$_{50}$-bark: 5.7 µg/mL).

Lipoxygenase (LOX) inhibitory activity in extracts of *E. senegalensis*

Extracts of *E. senegalensis* DC showed a dose-dependent inhibition of 12-LOX (Figure 3). EtOAc extracts were the most active (IC$_{50}$: 4.95 ± 1.12). These presented inhibitory potentials far exceeding those of DCM extracts (Table 3).

Inhibitory effects of DPPH and 12 - LOX in extracts of *E. senegalensis* DC

We have observed (Table 4) that stem bark extract in EtOAc presented the best LOX inhibitory effect (IC$_{50}$-LOX: 4.95 ± 1.12) and a moderate radical-scavenging effect (IC$_{50}$-DPPH: 11.4 ± 1.3). However, root extract in EtOAc was as well radical-scavenging (IC$_{50}$-LOX: 7.21 ± 2.31) as inhibitory of the 12-LOX of soybean (IC$_{50}$-DPPH: 7.27 ± 0.13).

DISCUSSION

Extractions and phytochemicals

Dichloromethane has given higher yields of extraction than ethyl acetate extracts. The exhaustion of the stem bark by dichloromethane gave 4.24% as extraction yields higher than those found by Togola et al. (2009) (3.9%) who used soxhlet.

Steroids, triterpenes and coumarins were detected in all parts of the plant. Flavonoids and tannins were most found in stem bark than in roots. Our results are consistent with those of many authors.

Wanjii et al. (1994) isolated many phenylsoflavones and many triterpen compounds in methanolic extracts. Nacouima et al. (1996) indicated the presence of coumarins, flavonoids, saponins and steroids in the leaves, roots and bark of *E. senegalensis*. Saidu et al. (2000) characterized flavonoids, tannins and saponoside in macerated aqueous stem bark.

Our study did not highlight the presence of alkaloids in the studied parts of the plant. These results contrast with those of Nacouima et al. (1996) and Saidu et al. (2000) who indicated the presence of alkaloids in the bark, roots, leaves and flowers. The differences may be explained by the plant material and the method used. Indeed, for the screening of phytochemicals, Saidu et al. (2000) used the tests according to Trease and Evans (1983) on macerate aqueous stem bark. Likewise, our method of extraction (successive exhaustion with 2 solvents) probably contributed to spread the small proportion of these alkaloids in different solvents, so that diluted; they were undetectable in our working condition as described by Ciulei (1982). In addition, our plant materials were not collected in the same geographic area. The variation in composition of phytochemicals may be a response of plants to factors such as climate conditions (Reynolds, 2002), the geological environment of crops sites (Gomes et al., 2007), the period of harvest, the enzyme responsible for equipment of the metabolic
Table 2. Phytochemicals characterized by colored method according to Cuilei et al.

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Phytochemicals</th>
<th>Stem bark</th>
<th>Roots</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dichloromethane</td>
<td>Steroids and triterpens</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>Flavonoids</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>Coumarines</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Ethylacetate</td>
<td>Flavonoids</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Tannins</td>
<td>++</td>
<td></td>
</tr>
</tbody>
</table>

Note: (+): low quantities; (++): high quantities.

Figure 1. Tannins, flavonoids, triterpens and steroids characterized by Thin Layer Chromatography method. Sb: stem bark; R: roots.

pathways of biosynthesis (Pieters et al., 2005) and the regulation of the expression of genes (Boudet, 2007). TLC indicated a predominance of catechic tannins over gallic tannins in all the parts of the plant. Flavonoids were the most abundant in stem bark extracts. Roots extracts contained more phenolic acids than flavonoids. Triterpens and steroids were characterized in all the parts of the plant.

Radical scavenging activity in extracts of *E. senegalensis*

DCM extracts of root and stem bark have globally shown the best dose-dependent radical scavenging effect (IC₅₀: root: 5.1 µg/ml and IC₅₀-bark: 5.7 µg/ml). These results are consistent with Soro et al (2005) who found (by autobiography to DPPH) that radical-scavenging effect
was more marked with extracts in DCM. Extracts in DCM are lipophilic; these might contain many fat-soluble polyphenols (flavonoids, tannins) or their aglycones, triterpens and sterols responsible for the strongest radical scavenging activity. In many studies (Karou et al., 2005; Brewer, 2011); authors link the radical-scavenging effect of plant extracts to polyphenols. The radical-scavenging activity of polyphenols is due to their redox property which would play an important role in the adsorption, the capture, the neutralization of free radicals or in the decomposition of peroxide (Karou et al., 2005). The flavonoid heterocycle (aglycone) contributes to antioxidant activity by the presence of a free 3-OH, and permits conjugation between the aromatic rings. The closed C-ring itself may not be critical to the activity of flavonoids, given that chalcones are active antioxidants (Brewer, 2011).

The analysis of the IC$_{50}$ values indicates that root extracts in EtOAc ($7.2 \pm 0.13 \mu g/mL$), despite containing less various polyphenolic compounds had a radical-scavenging potential. There is no relationship between the radical-scavenging effect and the quantitative composition in total polyphenols (Boudet, 2007). The ability to trap free radicals and radical-scavenging activity of polyphenols would depend on the arrangement of functional groups in relation to the structure of the nucleus. For more, according to (Brewer, 2011), the number and configuration of the hydroxyl group of donors
of hydrogen would constitute the major influences of the radical-scavenging potential of polyphenolic compounds. Our best results (7.2 ± 0.13, 5.76 ± 0.68 and 5.18 ± 0.06 µg/mL) compared to the effect of quercetin (IC50: 2.25 ± 0.001 µg/mL) taken as reference in our study remained low. Nevertheless, our crude extracts possess a non-negligible radical-scavenging potential, since quercetin is a purified compound. Free radicals are highly reactive forms. Oxidative damage to lipids (lipid peroxidation) disrupts the functioning of the membranes, causes deposits of oxidized fat in vessels (formation of atheroma plates) and will generate carcinogens derivatives. The oxidation of proteins can damage cell proliferation or defense signals, inhibit enzymes and be responsible for deposits of amyloidosis and fibrosis (Lobo et al., 2010). Radical attacks of the DNA will be sources of rupture and cell death, but especially of carcinogenic mutations (Lobo et al., 2010).

From the foregoing, our potentially anti-radical extracts may be interesting in the prevention or reduction of the pathogenesis of these diseases.

### Lipoxygenase (LOX) inhibitory activity in extracts of E. senegalensis

EtOAc extracts were the most active. These have presented inhibitory potentials far exceeding those of DCM extracts. Togola et al. (2009), for their part, have found the best LOX inhibitory effect in DCM extract from roots. Phytochemicals screening in EtOAc extracts has reported an abundance of flavonoids and tannins in stem bark and a scarce presence of flavonoid in roots. Thus, it would be possible that the presence in the bark of flavonoids and tannins, in a contributory way, is responsible for the strong inhibitory activity of lipoxygenase (synergy of action). These results are in line with other authors who have highlighted the inhibitory effects of polyphenols (tannins, flavonoids) on LOXs (Hu et al. 2006; Togola et al., 2009).

LOXs are a family of enzymes with a non-heme iron responsible for stereo and region specific dioxygenation of polyunsaturated fatty acids that contain a 1, 4-pentadiene motif (Mashima et al., 2015). Derivatives such as 12-hydroperoxy-eicosatetraenoic acid (12-HPETE), 12-Hydroxy-eicosatetraenoic (12-HETE), leukotrienes and lipoxins are involved in cancer, cardiovascular disease, asthma, rheumatism (Wisastra et al., 2014). Thus, inhibition of these enzymes can help reduce the occurrence of these diseases.

### Inhibitory effects of DPPH and 12 - LOX in extracts of E. senegalensis DC

The extract of stem bark in EtOAc in which abundant flavonoids have been characterized, presented the best LOX inhibitory effect without radical-scavenging effect (IC50-LOX: 4.95 ± 1.12 versus IC50-DPPH: 11.4 ± 1.3). Wangensteen et al. (2006) have shown that the DPPH scavenging and the LOX inhibition were not linked. The involvement of proton donation from these active compounds may be of less importance for LOX inhibition than for DPPH radical scavenging. In fact, this lack of correlation may suggest that LOX inhibitory compounds act by directly binding to the enzyme. The root extract in EtOAc was as well radical-scavenger as 12-LOX inhibitor (IC50-LOX: 7.21 ± 2.31 versus IC50-DPPH: 7.27 ± 0.13). These results suggest that LOX inhibition would have required a donation of hydrogen atom. Indeed, in the reaction of peroxidation by LOX, polyunsaturated fatty acid must lose a hydrogen atom then rearrange before setting the radical oxygen. The gift of proton would be to compensate for this loss of hydrogen and then block the stage of dioxygenation. TLC had shown that this extract contained phenolic acids that could explain this activity.
Conclusion

Our study focused on *Erythrina senegalensis DC (Fabaceae)*, a medicinal plant of Burkina Faso. Roots and stem bark of the plant concentrate flavonoids, tannins, coumarins, and sterols and triterpenes. DCM extracts showed powerful anti-radical (DPPH) effects without 12-LOX inhibitory effect. However, EtOAc extracts possess both types of effects with a predominance of the inhibitory effect of 12-LOX of soybean. We show in this study that extracts of *E. senegalensis DC (Fabaceae)* have an antioxidant potential and provide interesting data for the research of purified phytochemicals or for the development of phytomedicines for the prevention of cancer diseases, cardiovascular diseases or chronic inflammatory diseases.

Conflict of interest

The authors have not declared any conflict of interest

ACKNOWLEDGEMENTS

This study was supported by Conférence Episcopale Italienne (CEI), Union Economique Monétaire Ouest Africaine (UEMOA) for FS, CG, JBN, JS and Health’s Ministry of Burkina Faso for FS and IPG. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

REFERENCES


